REMARKS

Claims 1, 3, 11-17, and 36 are herein amended, claims 10 and 18-35 and 37-56 are cancelled without prejudice, to be submitted and prosecuted at a later time (claims 10 and 21-22 were cancelled in a previous response), claims 57-58 were not entered, and claim 59 is new. In light of the amendments and arguments submitted herewith, Applicants respectfully request reconsideration of pending claims 1 – 9, 11-17 and 36 and new claim 59.

Amendments to the Specification

The first paragraph (lines 3-6) on page 1 of the specification ("Cross Reference to Related Applications") has been amended to replace "gains priority from ..." with "claims priority from ..." Applicants submit that the language now properly reflects the intent of the Applicants to claim priority to the recited provisional applications.

The first paragraph of page 18 (lines 1-5) of the specification is also amended to remove the improper reference to a later page number in the specification. The new language now merely references the example protocol as being provided "below."

Applicants respectfully submit that no new matter is introduced with these amendments.

Amendments to the Claims

Claim 1 is amended to delete, in the "introducing" step the phrase ~~a transfection preparation comprising~~ and to add the limitation human embryonic stem before "cells", to indicate that the polynucleotide is introduced into hES cells. Support for this limitation is found throughout the specification and in the examples. Other minor changes to format and grammar are made, for aesthetic and clarity reasons.

Claim 3 is added to include a verb to describe the protein expression in line 2, for grammatical reasons.

Claim 11 is amended in the "introducing" step in like manner as claim 1. Support for this limitation is as indicated for claim 1. Claim 11 is also amended to remove the improper Markush format since only a single transfection reagent is listed.

Dependent claims 12-17 are amended to properly begin with "The method" rather than "A method."

Claim 36 is amended to require a "*substantially pure stably transfected* population of *pluripotent human embryonic stem cells*, wherein said cells are modified to contain a gene expression altering sequence of DNA." Support for the amendment is found in the specification, among other places, on p. 4 lines 32-34 and p. 5, lines 32-34.

New dependent claim 59 is added to include a selection and verification step to the transfection method of claim 1. Support is found in the specification on p. 13, line 1 through p. 15, line 4.

No New Matter

Applicants respectfully submit that no new matter has been added with any of the amendments to the claims detailed above.

Cancelled Claims

Claims 10, 22, and 23 were previously cancelled without prejudice, and withdrawn claims 18-21, 24-35 and 37-47, drawn to a non-elected invention, are cancelled herein. In addition, product claims 36 and 48-56 are cancelled. Thus, claims 18-56 are now cancelled. The Applicants reserve the right to prosecute any or all such claims in a follow-on application.

Claim Objections

Applicants submit that as amended, claims 3, 11 and 12-17 are no longer objectionable.

Claims 57 and 58, whose status is identified as "not entered" with no recitation of the text, are proper as written. According to MPEP § 714 C(A), "The current status of all of the claims ... must be given. Status is ...by one of the following: (original), ... or (not entered). ... Canceled and not entered claims must be listed by only the claim number and status, without presenting the text of the claims." Therefore, Applicants respectfully submit that the status identifier and absence of listing for claims 57 and 58 are proper.

Prior Art Rejections

1. Indefiniteness

In light of the cancellation of the claims at issue (claims 49-56) the rejections based on indefiniteness are moot.

2. Anticipation

Claims 18-35 And 37-56 are now cancelled. Applicants thank the Examiner for withdrawing the anticipation rejections of claims 1-4, 6 and 9-13 in the Office Action of 11/17/04. In light of the cancellation of claims 18-35 and 37-56, the remaining rejections cited in the Office Action based on anticipation are therefore moot, with the exception of claim 36. Regarding claim 36, it is now amended to require a "*substantially pure stably transfected* population of *pluripotent human embryonic stem cells*, wherein said cells are modified to contain a gene expression altering sequence of DNA." Because the claim limits the product to a *population*, and because the population must be *substantially pure*, *stably transfected* and pluripotent, applicants respectfully submit that Smith does not anticipate the presently claimed invention. Smith does not obtain a *stably transfected*, substantially pure population of pluripotent hES cells using the transfection methods disclosed. Although Smith may allow someone to select and generate, after transfection, a population of hES cells with a gene altering sequence, at the time of the initial transfection, the methodology of Smith does not result in a population of *substantially pure*, *stably transfected* pluripotent hES cells containing a gene expression altering sequence.

2. Non-obviousness

Applicants would first like to thank the Examiner for withdrawing the obviousness rejection of claims 1- 4, 6, 10, 36, 48, 52, 54 and 56 based on the combination of Thomson and Bradley.

The Examiner has maintained the rejections of the pending claims for reasons of obviousness based on an initial combination of Smith et al. and Fasbender et al. (cited in previous actions), in combination with various other references for certain dependent

claims (i.e. Myers for claims 5 and 14; Pascalo for claim 17; and Gibco BRL catalog for claims 8 and 9). However, the motivation to combine or modify Smith and Fasbender, the initial combination on which all the obviousness rejections are based, does not provide one skilled in the art with a reasonable expectation of success and does not teach all the elements of independent claims 1 and 11.

Smith is not enabling for any transfection protocol except electroporation,[1] and Fasbender is drawn to using a cationic polymer or a cationic lipid in combination with an adenovirus to *infect* human epithelia or nasal epithelium of cystic fibrosis in *mice* (see Abstract). But as unexpectedly discovered by Dr. Benvenisty and others, electroporation does not work in hES cells because electroporation kills most hES cells during attempted transfection.

As attested by Dr. Benvenisty in his Declaration of April 18, 2005 (Declaration 2) at para. 3, "Since its advent, electroporation has proven to be the method of choice for transfection, from *E. coli* to mammalian systems. Even today, a biotechnology company called Genetronics, Inc. that is developing gene therapy commercially, and thus in need of efficient means for introducing genes into human cells, uses electroporation as the transfection means of choice, and gives a little tutorial on its website of the advantages of electroporation over other standards means of transfection, and recites the disadvantages of those other transfection methods. The Genetronics, Inc. website (http://www.genetronics.com/sciencegenedelivery.htm) (see attached Exhibit B) states:

> Gene therapy is the treatment of genetic and acquired diseases through the insertion of genes into cells. ... However a gene is a large, chain-like molecule, and difficult to get into a cell. The method frequently being used today is to attach the gene to a disabled virus ...[such as a] retrovirus whose properties have been extensively studied. Retrovirus-mediated transfer *is viewed with some misgivings*, since it brings the gene of the virus into the cells along with the possibility of mutation. ....
>
> Other methods of gene delivery-such as liposomes, cationic lipids, microinjection or biolistic gun-typically yield *inefficient gene transfer and expression* or are inconvenient, invasive and costly.

[1] Although Smith lists other means for transfection, and discusses transfecting in mammalian ES cells other than murine ES cells, no examples or protocols for any system/mammal is provided other than transfection of murine ES cells by electroporation. Thus, it is Applicants' position that Smith is only enabling for transfection by electroporation in murine ES cells.

> These methods and viral vectors *can cause immune complications* or limit the size of the DNA being delivered. Electroporation, however, poses few complications and is applicable to a wide variety of targets." (emphasis added)."

However, contrary to the experiences of researchers in almost every other system to date, electroporation surprisingly proved to be a poor choice for transfecting human ES cells. Even Dr. Benvenisty originally believed that electroporation would be the transfection method of choice for hES cells, given its success in mouse ES cells. As pointed out by Dr. Benvenisty "...until our successful transfection of hES cells using chemical transfection, those in the field, including my laboratory, expected that electroporation would be the transfection protocol of choice for human ES cells, based on past success in transfecting numerous organisms as diverse as bacteria and mice, including previous successful transfection of mouse ES cells using electroporation. (see para. 5, Declaration 2).

But as explained by Dr. Benvenisty, "electroporation just does not work as well in hES cells as it does in almost every other organism, as discovered by Dr. Benvenisty and soon by others. Even as late as 2003, as seen in a Technical Report by Zwaka and Thomson (*Nature Biotech* (2003), vol. **21**, pp. 319-321– submitted with Declaration 1 as Exhibit C) researchers were reporting lower than expected transfection rates in hES cells when electroporation was used. Zwaka states that "For human ES cells, the best chemical reagents yield stable (drug-selectable) transfectants at rates of about 10^{-5}; mouse ES cell electroporation procedures yield substantially lower rates." The electroporation transfection rate in that article was reported as being only about ~10^{-7} when mouse electroporation protocols were adapted and applied for transfection of hES cells (see Zwaka, p. 319, col. 2, first para.)" (see Declaration 2, para. 6).

Dr. Benvenisty goes on to state that "in this respect, the entire field was initially taken by surprise" but that by the time of his laboratory's successful efforts at stably transfecting hES cells, "it had become clear to those of us working in the field and trying to successfully transfect human ES cells ... that electroporation did not introduce DNA into hES at rates or levels high enough to do meaningful research with genetically altered human ES stem cells" (*id*)

Moreover, Dr. Benvenisty explains that "Figure 1 in the application shows just how inefficient electroporation in hES cells actually is. At first glance, it appears from Fig. 1 of the application that only transfection in the presence of ExGen 500™ resulted in transfection rates an order of magnitude better than those seen with electroporation in hES cells. In addition, it appears that transfection with other transfection reagents, e.g. LipofectAMINE Plus™ or FuGENE™, resulted in only marginally better or even worse transfection rates than seen with electroporation." (see Declaration 2, para. 7)

However, the apparent results shown in Figure 1 belie the reality of the results. Dr. Benvenisty continues that, "The values shown in Figure 1 of the application are inaccurate reflections of the actual transfection rates observed when using chemical transfection in the presence of the transfection reagents, as compared to transfection rates using electroporation. That is because my laboratory determined that electroporation unexpectedly killed almost all of the hES cells during the electroporation procedure, possibly because hES cells are much more fragile than other cells and cannot withstand the electroporation procedure. Thus, the transfection rate depicted for electroporation in Fig 1 is misleadingly high since it reflects a rate based on almost ten times as many original cells as the rates depicted for chemical transfection in the presence of the indicated transfection reagents" (see para. 8 Declaration 2).

More importantly, the rejections in the Office Action are based on an underlying assumption that chemical transfection in the presence of transfection reagents is obvious given that others had predicted that electroporation would prove successful for transfecting human ES cells, that there was no reason to believe these predictions would not prove to be true, and so other transfection methods should work as well. But Dr. Benvenisty clarifies that "Electroporation may be successful for transfecting murine ES cells, but not for transfecting human ES cells. Because of the substantial differences which exist between mouse and human embryonic stem cells - see the Table in Declaration 1 and discussions of same in Kaufman et al. (*Proc. Natl. Acad. Sci. USA*, Vol. **98**, No. 19, pp. 10716 – 10721, (2001) – attached in Declaration 1 as Exhibit D) – we were not able to use electroporation to transfect hES cells. Unexpectedly, electroporation killed most of the hES cells during the transfection procedure itself. Therefore, my laboratory started looking for a different transfection methodology for hES

cells, which is what ultimately led us to the claimed invention. The fact that we had to look for alternatives to electroporation prior to 2001, and that Zwaka et al. reiterated in 2003 that transfection using electroporation protocols as performed with mouse ES cells did not work for introducing DNA into hES cells, is more evidence that the devising of a transfection protocol for hES cells was not obvious" (see Declaration 2, para.11)

Thus, although Applicants did show very minimal transfection using electroporation (see Figure 1 of the application), they also concluded that because electroporation killed most hES cells, the protocol was too harsh and the rates were too low to be useful.

And as detailed by Dr. Benvenisty, "The work in our laboratory, as published in Eiges et al. (see exhibit B, Declaration 1) shows that chemical transfection using the transfection reagent ExGen 500™, a polycationic non-lipid polymer, resulted in transfection rates an order of magnitude better than achieved using electroporation, LipofectAMINE Plus™ or FuGENE™ when the was used (see Eiges, Figure 1) and that the quantitative transfection rate we achieved was ~10^{-5} compared to ~10^{-7} for electroporation (see above). What is not apparent from Figure 1 is that we had to start with about 10x as many cells for the electroporation data to measure the rates we observed for transfection with electroporation. Thus, our transfection methods using cationic polymers yielded transfection rates more than an order of magnitude better than electroporation in any system" (see Declaration 2, para 12).

Taken in the context of the above arguments – that even the other chemical reagents shown in Figure 1 transfect hES much better than electroporation since the values show for electroporation do not reflect the poor viability and 10-fold higher starting number of cells for electroporation - the skilled person would not start with the teachings of Smith and apply the electroporation protocols for transfecting mouse ES cells disclosed therein to human ES cells, because the electroporation transfection process would inevitably fail in human ES cells. Human ES cells are extremely fragile and the finding that the electroporation process itself kills most hES, resulting in extremely low rates of successful transfection, does not provide motivation for combining/modifying the cited prior art of Smith and Fasbender because there is no reasonable expectation for success. Those skilled in the art would understand the lack of a reasonable expectation of

success and so would not be motivated to combine the teachings of Smith with the other cited references to arrive at the present invention.

Second, even if combined, the combination does not teach all the elements of claim 1 or claim 11. As amended, Applicants respectfully submit that claims 1 and 11 no longer encompass electroporation as a means of transfection, or the use of adenovirus during (or for) transfection. Claims 1 and 11 specify that a polynucleotide is introduced in hES cells "by transfection in the presence of " either "at least one transfection reagent selected from the group consisting of ..." or "a cationic polymer agent" (see claims 1 and 11, step (ii), respectively). Transfection in the presence of at least one transfection reagent excludes electroporation and adenovirus. Electroporation is not performed in the presence of any transfection reagents because it is a mechanical means for transfecting, not a chemical means, and adenovirus is a virus that infects the cell to incorporate the polynucleotide of interest and not considered a transfection reagent (see discussions above).

As stated by Dr. Benvenisty "those skilled in the art would not perform electroporation in the presence of transfection reagents. A protocol that requires transfection in the presence of transfection reagents, as claimed in our invention, is understood to be a chemical method of transfection. Electroporation is a mechanical means for transfection, not a chemical means, and does not require additional transfection agents to facilitate the entry of nucleic acids into the cell because the applied electric current acts to disrupt the cell membrane and allow influx of nucleic acids into the cell, so no added transfection reagents are needed." (see Declaration 2, para. 13).

Using adenovirus in conjunction with transfection of hES cells is also problematic because as explained by Dr. Benvensity "adenovirus transfects cells by infecting them, and infecting hES cells with a virus is potentially too dangerous because of the possibility of introducing or activating oncogenes for it to be considered as a viable means for transfection in hES cells. In short, adenovirus infection is not compatible with the goal of obtaining stably-transfected hES cells for later therapeutic use" (see Declaration 2, para. 14).

As with Smith, there is no motivation to modify Fasbender to eliminate the adenovirus from the transfection protocol because Fasbender specifically states that

"[n]onviral cationic vectors ... do not catalyze the subsequent steps in gene transfer" (see Abstract, emphasis added). According to MPEP §2141.02, the "prior art must be considered in its entirety, i.e. as a whole, including portions that would lead away from the claimed invention" (as paraphrased from *W.L. Gore & Associates, Inc. v. Garlock, Inc.*, 721 F.2d 1540 (Fed.Cir. 1983)). Thus, Smith and Fasbender each individually teach away from the claimed invention, and together, the combination only decreases the likelihood of success.

For the same reasons, independent claims 1 and 11, and dependent claims therefrom, are not obvious in light of Smith and Fasbender combined with either Myers, Pascalo, or the Gibco-BRL catalog. Because the core rejection is based on the combination of Smith and Fasbender, and because these is no expectation of success and the combination does not teach all the elements of the claimed invention even if made, rejections for dependent claims relying on Smith and Fasbender with additional secondary references fail for the same reasons as detailed above.

Myers, Pascolo and the Gibco-BRL catalog are directed toward the teaching of specific fluorescent proteins, knockout genomic sequences, and the transfection reagent LIPOFECTIN®, respectively, but do not provide any teaching about transfection of human embryonic stem cells. Thus, even when combined with Smith and Fasbender there is still no expectation of success and all of the claim limitations of the present invention are still not taught or suggested by the combinations.

In summary, the *prima facie* case of obviousness fails primarily for lack of a reasonable expectation of success because of the unexpected results demonstrated by Applicants using chemical means for transfection compared to electroporation, contrary to the accepted assumption in the field that electroporation would be the transfection means of choice in hES, like it is in nearly all other systems where it has been used. Without the impermissible use of hindsight, one skilled in the art would not modify the teachings in the prior art of stem cell research – a hugely unpredictable field – to substitute chemical transfection, believed to be far inferior to electroporation as a means for transfection, for electroporation, the transfection means of choice for those skilled in the art. The combinations/modifications would not be made because those skilled in the

art would not expect better results using chemical transfection than the meager success seen with electroporation and hES cells.

The *prima facie* case also fails secondarily because the combinations do not teach all of the elements of the presently claimed invention. The Examiner has cited a multitude of combinations to attempt to establish a *prima facie* case of obviousness against the presently claimed invention, but the combinations do not hold up.

For all the foregoing reasons, Applicants respectfully submit that the pending claims are not obvious in light of the cited prior art. Reconsideration of the claims and withdrawal of the obviousness rejections are therefore requested.

CONCLUSION

In view of the arguments and amendments presented, Applicants respectfully submit that all pending claims are now in condition for allowance. Reconsideration of the claims and a notice of allowance are therefore respectfully requested.

Applicants believe that a two-month extension of time is required and submit a petition for a two-month extension with this response, along with a check for $225 to cover the two-month Extension Fee. In the event that any additional fees are required for the timely consideration of this application, please charge deposit account number 19-4972.

Respectfully submitted,

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